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of hyperferritinemia in the oldest individual identified with the R178G mutation can either be interpreted as incomplete penetrance or as evidence against R178G being a disease causing mutation. This raises an important question: When do we consider a genetic variant disease causing?

In the age of genome wide association studies and whole genome sequencing, this question becomes increasingly important. The frequency of a sequence variant does not necessarily reflect the functional importance because even in ferroportin related iron overload, common polymorphisms in *SLC40A1*, such as Q248H, have been reported as a strong risk factor for hyperferritinemia [1]. On the other hand, V72F represents a rare sequence variant that may still be of little functional consequence [2].

Taken together, a genetic variant is likely to be disease causing if the mutation segregates with the disease within a family and is not present in an ethnically matched population. In silico studies can provide further evidence for the functional consequence of genetic variations, which – for R178G – supports the notion that this mutation is disease causing [3]. Finally, functional and structural studies of the mutant protein in cells or knock-in animals have not been reported for R178G. Hence, the question remains whether R178G is disease causing or not. This uncertainty is paradigmatic for ferroportin disease and is expected to increase, because it remains to be resolved how to analyze millions of sequence variants detected by next generation sequencing technology [4] that, at the threshold of entering clinical practice, can be interpreted and functionally analyzed.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Roman Mayr
Heinz Zoller*
Medical University of Innsbruck,
Department of Medicine II, Gastroenterology, and Hepatology,
Anichstrasse 35,
A-6020 Innsbruck,
Austria
**E-mail address:* heinz.zoller@i-med.ac.at (H. Zoller)

Distinct, alcohol-modulated effects of *PNPLA3* genotype on progression of chronic hepatitis C

To the Editor:

Recent large-scale genetic studies in patients with chronic hepatitis C (CHC) provide evidence for a significant role of host genetic factors in its natural course [1], response to therapy [2], and incidence of therapy-related adverse effects [3]. Recently, a variant (rs738409 G>C) within the gene coding for patatin like phospholipase domain containing-3 (*PNPLA3*) was found associated with liver fat content and progression of non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) [4,5]. PNPLA3 modulates storage of triglycerides in hepatocytes by a yet unknown mechanism possibly related to lipid hydrolysis or cross-talk with other mechanisms governing lipid storage with consecutive "lipid trapping" in those patients carrying genotype *PNPLA3* rs738409 GG [6]. Although there is agreement on the important functional role of viral epitopes in the evolution of steatosis in CHC [7,8], host genetic factors, such as *PNPLA3* variation, could further modulate the accumulation of liver fat. In this issue of the *Journal of Hepatology* and elsewhere, two independent groups of researchers from distinct European regions demonstrate a significant association of *PNPLA3* rs738409 G with fibrosis, steatosis, and inflammation in patients with CHC [9,10]. Cai *et al.* included 626 patients from the Swiss Hepatitis C Cohort Study and found a significant association of *PNPLA3* rs738409 GG carriage with steatosis of any degree in genotype non-3 patients, whereas no impact of this variant on the stage of liver fibrosis was observed [9]. Regarding this lack of association, a stratified analysis of rs738409 towards steatosis and fibrosis in obese and/or alcoholic CHC patients would be interesting, because BMI and alcohol consumption were independently associated with the degree of steatosis in this cohort.

We ourselves tested a possible association of *PNPLA3* rs738409 with the severity of steatosis and fibrosis in 605 previously untreated Caucasian patients with CHC from three German University centres (Table 1). Assuming a recessive model controlling for sex, age, BMI, and viral genotype 3, both the presence of steatosis (grades 1–3 versus grade 0) and cirrhosis (METAVIR stage 4 versus stage <4) was significantly associated with genotype *PNPLA3* rs738409 GG. Upon stratification of alcohol intake into abstainers (<30 g/day) and at-risk drinkers (>30 g/day), a distinct risk pattern emerged: while genotype rs738409 GG was primarily relevant for steatosis in abstainers, its association with pre-treatment fibrosis was restricted to at-risk drinkers. Our data are in line with the large study from Valenti and coworkers from Italy [10], who found a significant association of genotype *PNPLA3* rs738409 GG with steatosis and fibrosis, no-response to antiviral treatment, and the occurrence of hepatocellular carcinoma.

Together, data from these three independent studies lend strong support for a significant role of *PNPLA3* rs738409 not only

Table 1. Logistic regression analysis results for the association of *PNPLA3* SNP rs738409 with the presence of steatosis and cirrhosis in 605 German patients with CHC.

	Recessive		Additive		n
	OR (95% CI)	*p*	OR (95% CI)	*p*	case/control
Steatosis*					
Total	5.53 (1.55-19.75)	0.009	1.86 (1.29-2.69)	0.001	273/169
Abstainers[a]	12.61 (1.48-107.7)	0.021	2.07 (1.25-3.44)	0.005	137/98
At risk drinkers[b]	2.61 (0.50-13.47)	0.253	1.74 (1.01-3.03)	0.049	136/71
Cirrhosis**					
Total	2.76 (1.22-6.25)	0.015	1.47 (1.03-2.09)	0.033	115/490
Abstainers[a]	1.65 (0.54-5.03)	0.376	1.16 (0.73-1.84)	0.528	73/262
At risk drinkers[b]	4.77 (1.39-6.38)	0.013	2.03 (1.16-3.56)	0.014	42/228

Logistic regression analysis using SPSS (PASW statistics 18) was performed for the association between the *PNPLA3* SNP and the presence of steatosis adjusting for age (45.8; 11.2), sex (47% females), BMI (25.0; 4.1), and viral genotype 3 (18.9%), and for the presence of cirrhosis adjusting for age (47.5; 11.6), sex (47% females), and BMI (25.1; 4.2) under a recessive and additive model of inheritance. In the analysis for the presence of steatosis, cirrhotic patients were excluded.
*Steatosis (grades 1–3 versus grade 0).
**Cirrhosis defined as fibrosis (stage 4 versus stage <4).
[a]Abstainers (<30 g alcohol per day).
[b]At-risk drinker (>30 g alcohol per day); % values for frequencies; values for continuous variables expressed as means with standard deviation.

in NAFLD and ALD, but also in CHC. Based on our dada, *PNPLA3* rs738409 appears to convey distinct effects towards either mere steatosis in non-drinkers, or progressive fibrosis of CHC in those who drink beyond amounts commonly considered as the threshold for harmful drinking.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Tobias Müller
Department of Hepatology and Gastroenterology, CVK, Charité Universitätsmedizin, Berlin, Germany

Stephan Buch
Department of Internal Medicine I, University Hospital Schleswig-Holstein, Kiel, Germany

Thomas Berg
Department of Hepatology and Gastroenterology, CVK, Charité Universitätsmedizin, Berlin, Germany
Department of Gastroenterology, University Hospital Leipzig, Leipzig, Germany

Jochen Hampe
Department of Internal Medicine I, University Hospital Schleswig-Holstein, Kiel, Germany

Felix Stickel
Institute of Clinical Pharmacology and Visceral Research, University of Bern, Switzerland
E-mail address: felix.stickel@ikp.uni